Figure 1

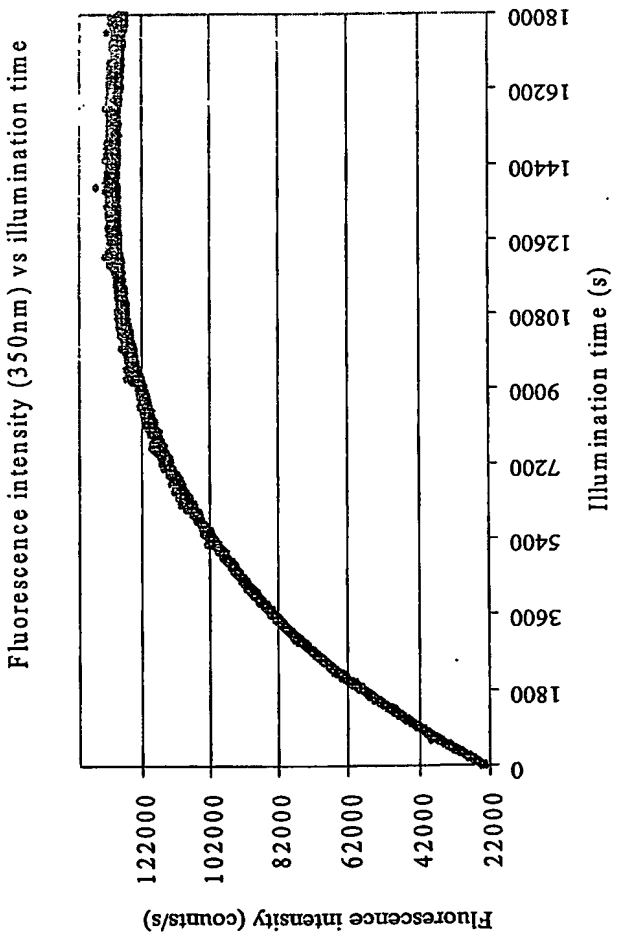
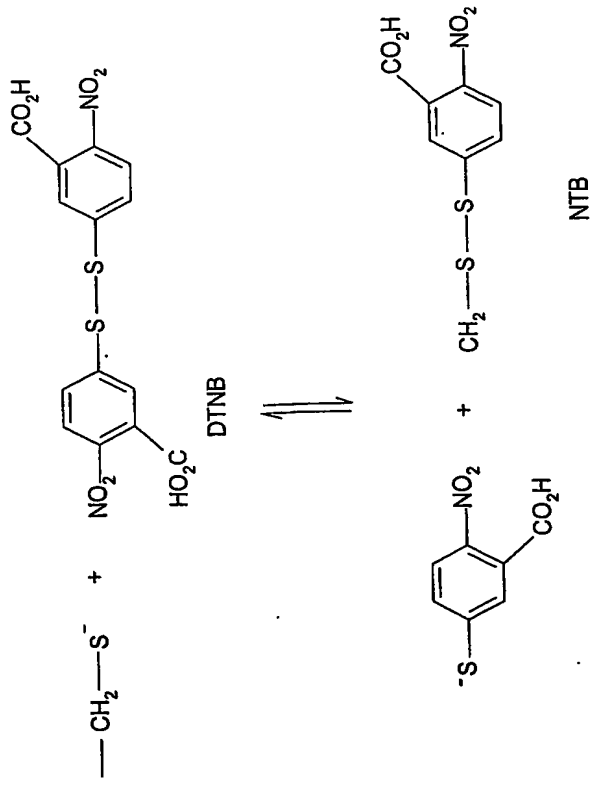

Fluorescence intensity (350nm) vs illumination time
Fluorescence intensity (counts/s)
122000
102000
82000
62000
42000
22000
Illumination time (s)
0
1800
3600
5400
7200
9000
10800
12600
14400
16200
18000

Figure 2



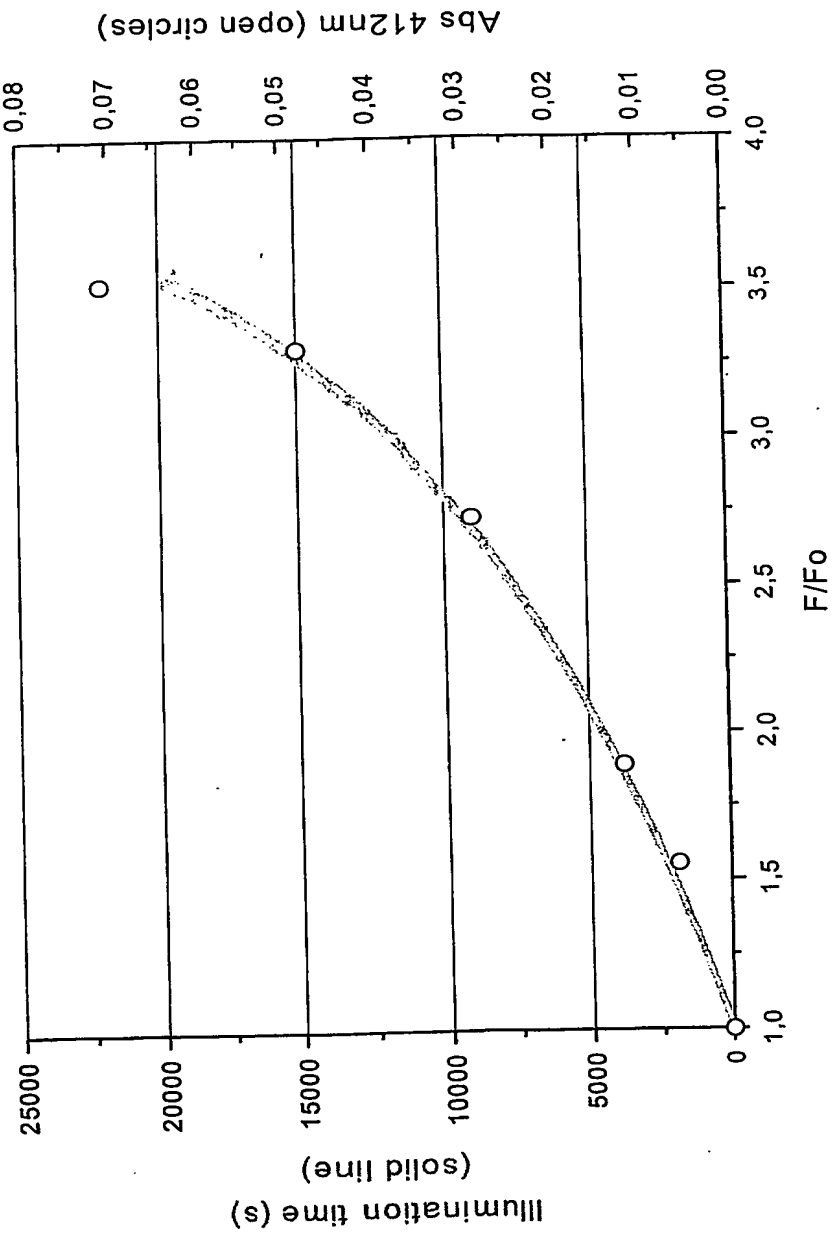

Abs 412nm (open circles)
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
4,0
3,5
3,0
2,5
2,0
1,5
1,0
F/Fo
25000
20000
15000
10000
5000
0
Illumination time (s)
(solid line)

Figure 3

Figure 4

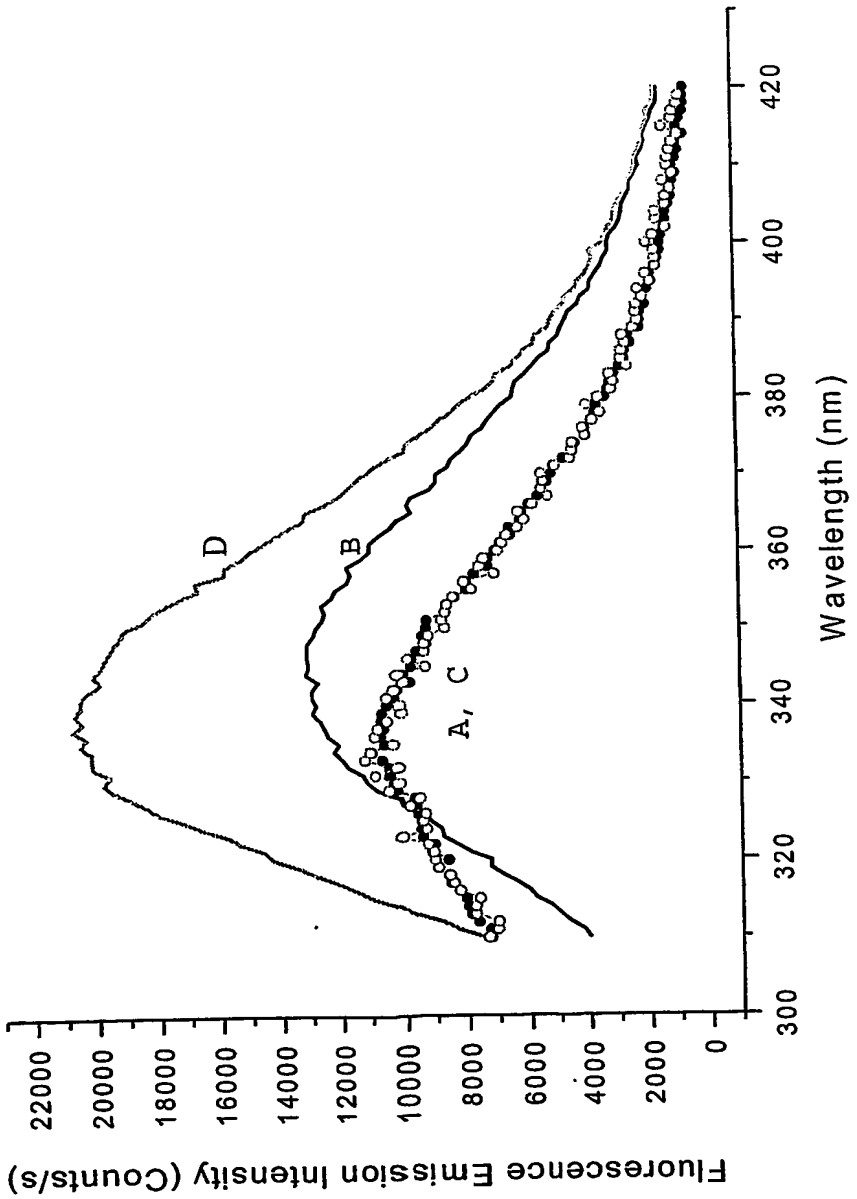

Fluorescence Emission Intensity (Counts/s)
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
22000
300
320
340
360
380
400
420
Wavelength (nm)
A, C
B
D

Figure 5

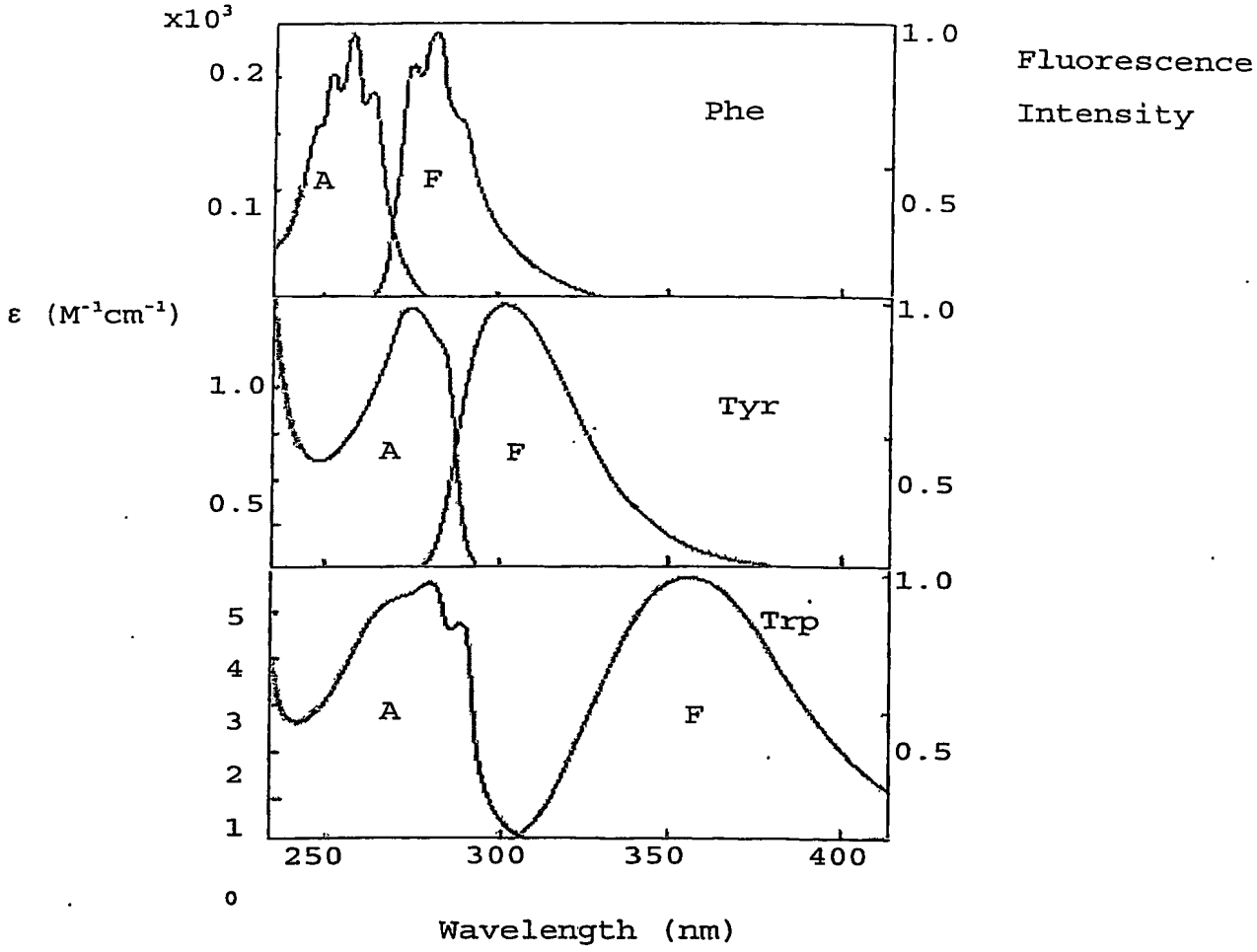

x10³
0.2
0.1
Phe
A
F
1.0
0.5
Fluorescence Intensity
ε (M⁻¹cm⁻¹)
1.0
0.5
Tyr
A
F
1.0
0.5
5
4
3
2
1
0
Trp
A
F
1.0
0.5
250
300
350
400
Wavelength (nm)

Figure 6

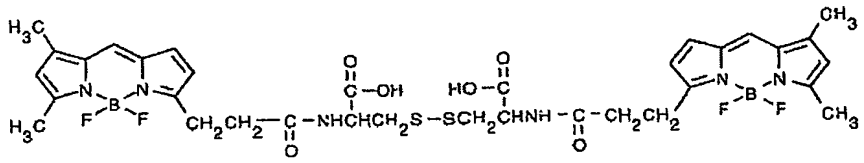
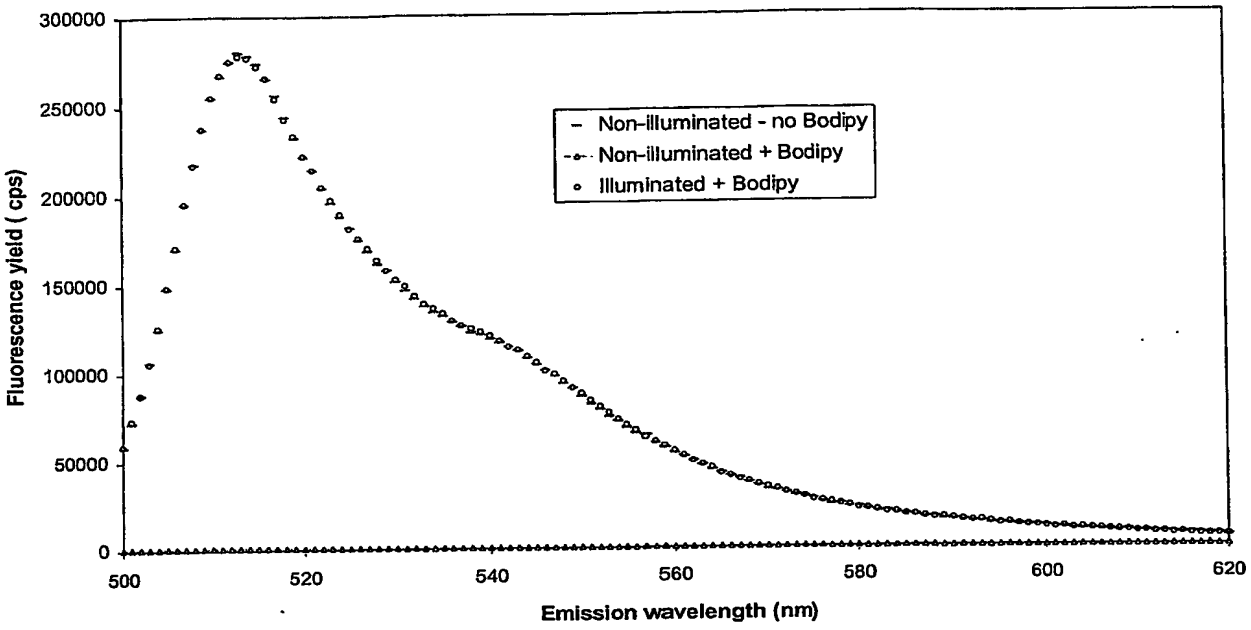


Figure 7

A


300000
250000
200000
150000
100000
50000
0
Fluorescence yield (cps)
500
520
540
560
580
600
620
Emission wavelength (nm)
Non-illuminated - no Bodipy
Non-illuminated + Bodipy
Illuminated + Bodipy

B

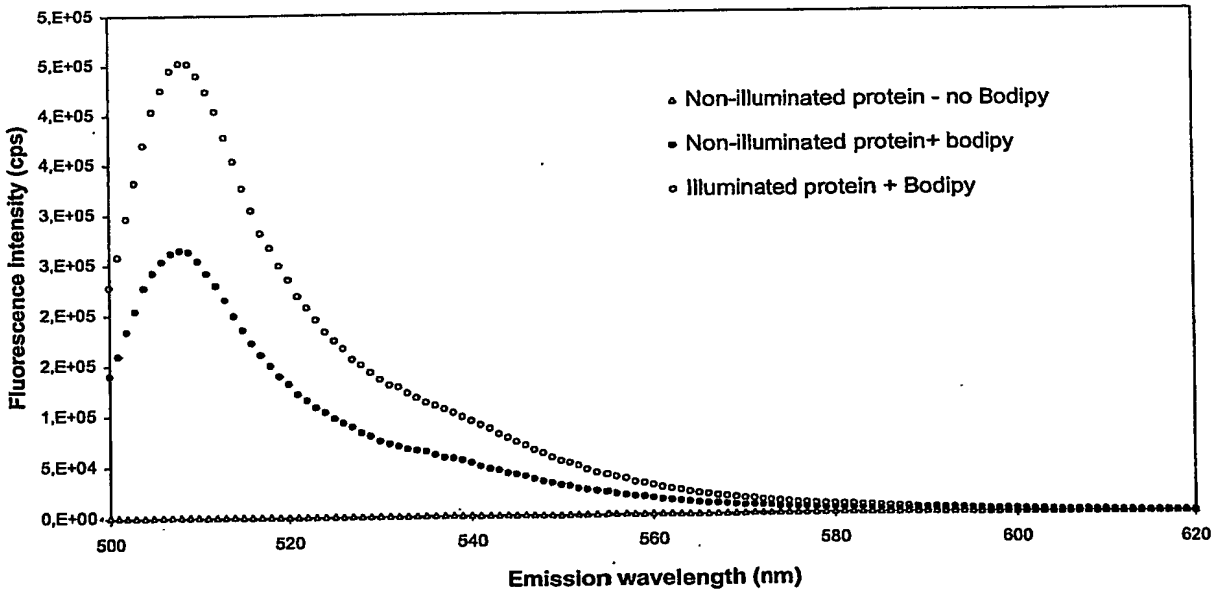
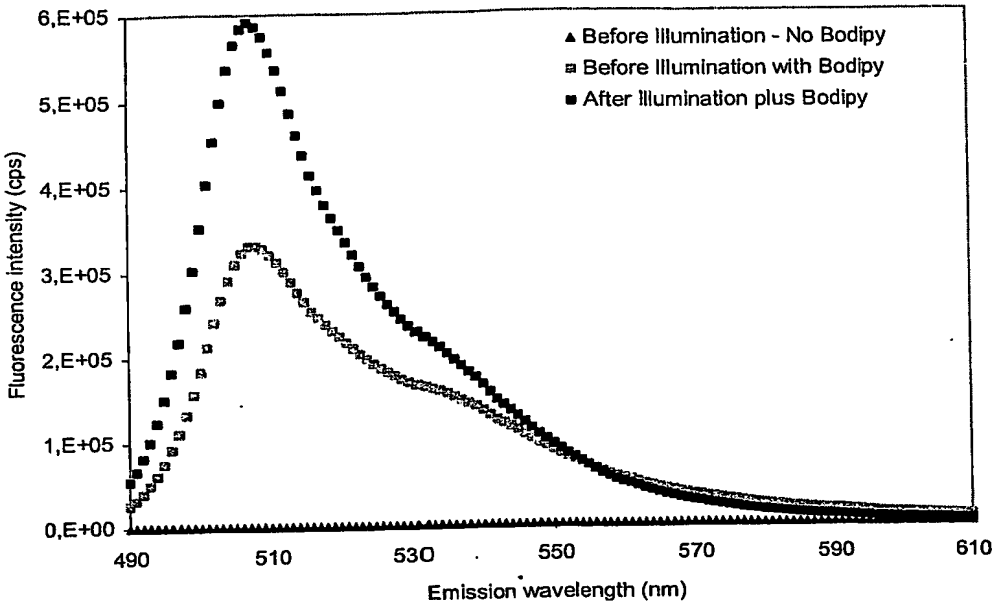
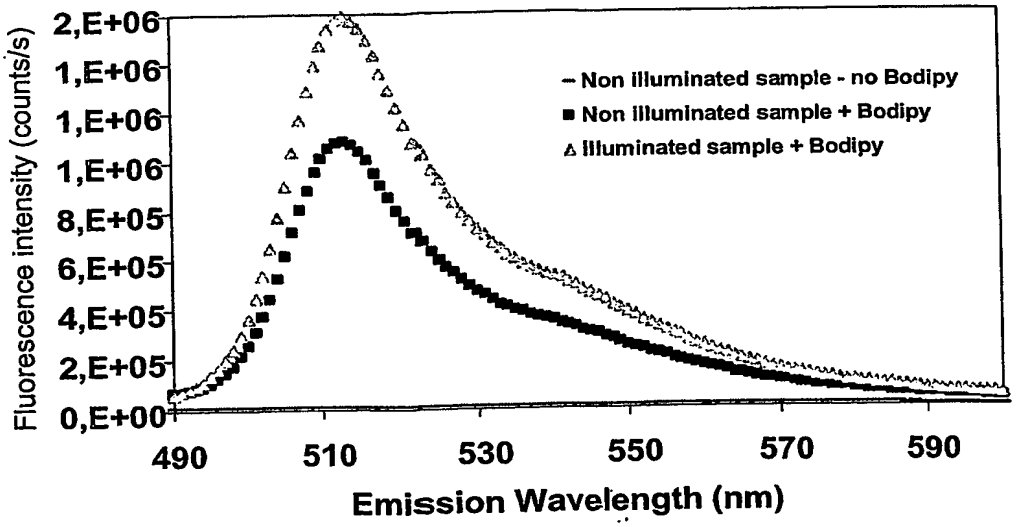

5,E+05
5,E+05
4,E+05
4,E+05
3,E+05
3,E+05
2,E+05
2,E+05
1,E+05
5,E+04
0,E+00
Fluorescence Intensity (cps)
500
520
540
560
580
600
620
Emission wavelength (nm)
Non-illuminated protein - no Bodipy
Non-illuminated protein+ bodipy
Illuminated protein + Bodipy

Figure 8

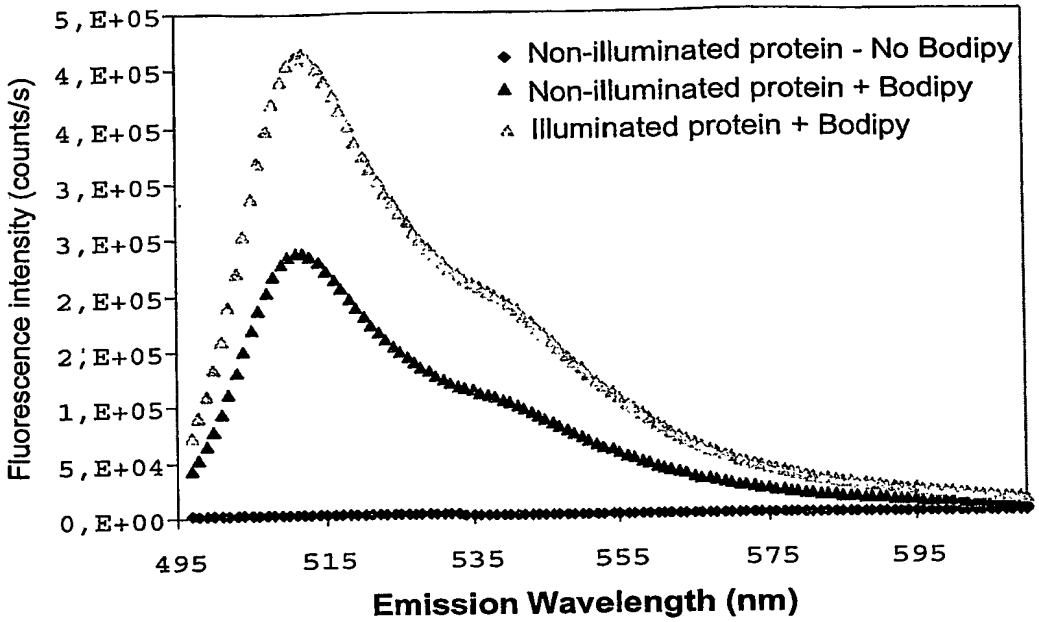
A


6,E+05
5,E+05
4,E+05
3,E+05
2,E+05
1,E+05
0,E+00
Fluorescence intensity (cps)
490
510
530
550
570
590
610
Emission wavelength (nm)
Before Illumination - No Bodipy
Before Illumination with Bodipy
After Illumination plus Bodipy

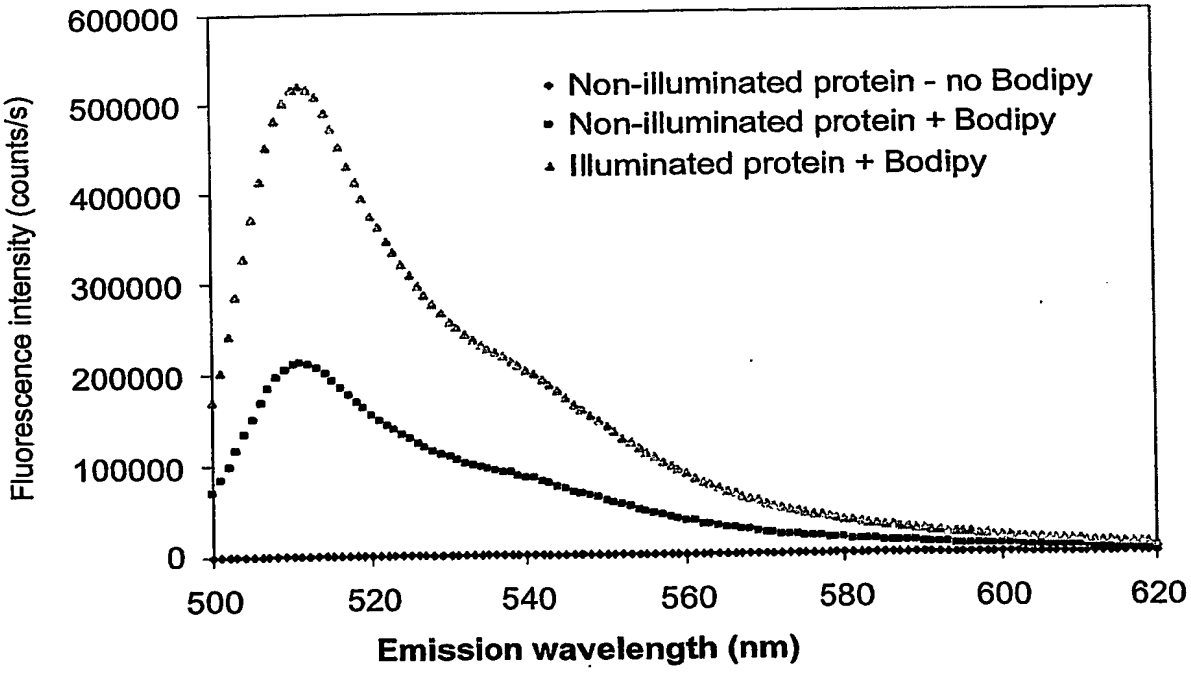
B


2,E+06
1,E+06
1,E+06
1,E+06
8,E+05
6,E+05
4,E+05
2,E+05
0,E+00
Fluorescence intensity (counts/s)
490
510
530
550
570
590
Emission Wavelength (nm)
Non illuminated sample - no Bodipy
Non illuminated sample + Bodipy
Illuminated sample + Bodipy

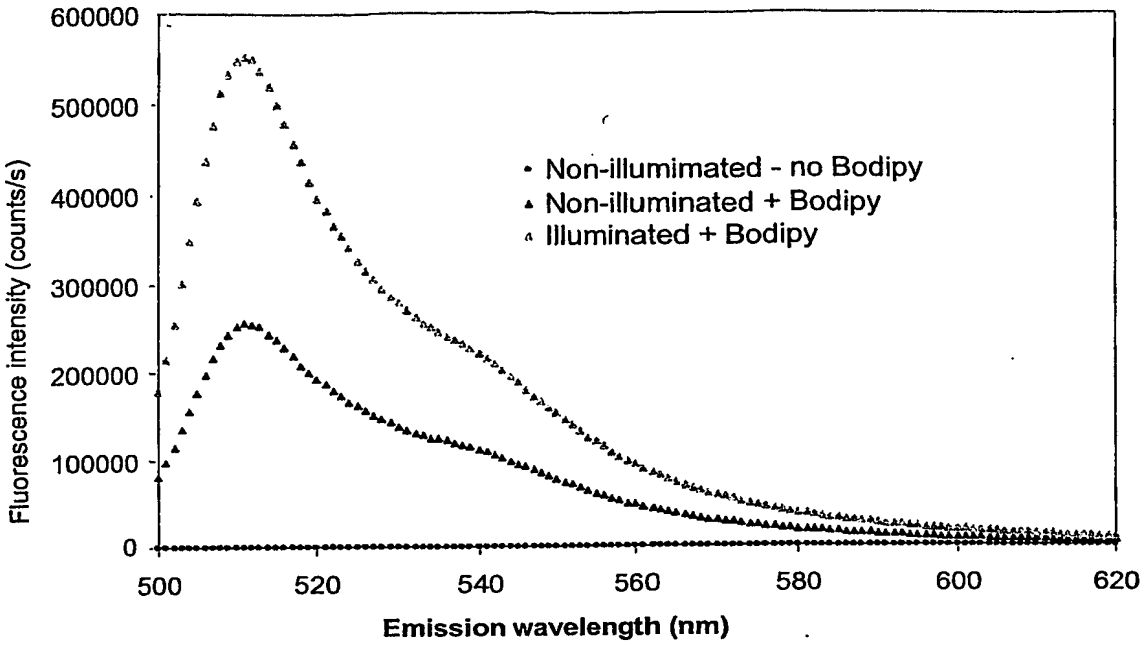
C


5,E+05
4,E+05
4,E+05
3,E+05
3,E+05
2,E+05
2,E+05
1,E+05
5,E+04
0,E+00
Fluorescence intensity (counts/s)
495
515
535
555
575
595
Emission Wavelength (nm)
Non-illuminated protein - No Bodipy
Non-illuminated protein + Bodipy
Illuminated protein + Bodipy

D


600000
500000
400000
300000
200000
100000
0
Fluorescence intensity (counts/s)
500
520
540
560
580
600
620
Emission wavelength (nm)
Non-illuminated protein - no Bodipy
Non-illuminated protein + Bodipy
Illuminated protein + Bodipy

E


600000
500000
400000
300000
200000
100000
0
Fluorescence intensity (counts/s)
500
520
540
560
580
600
620
Emission wavelength (nm)
Non-illumimated - no Bodipy
Non-illuminated + Bodipy
Illuminated + Bodipy

F

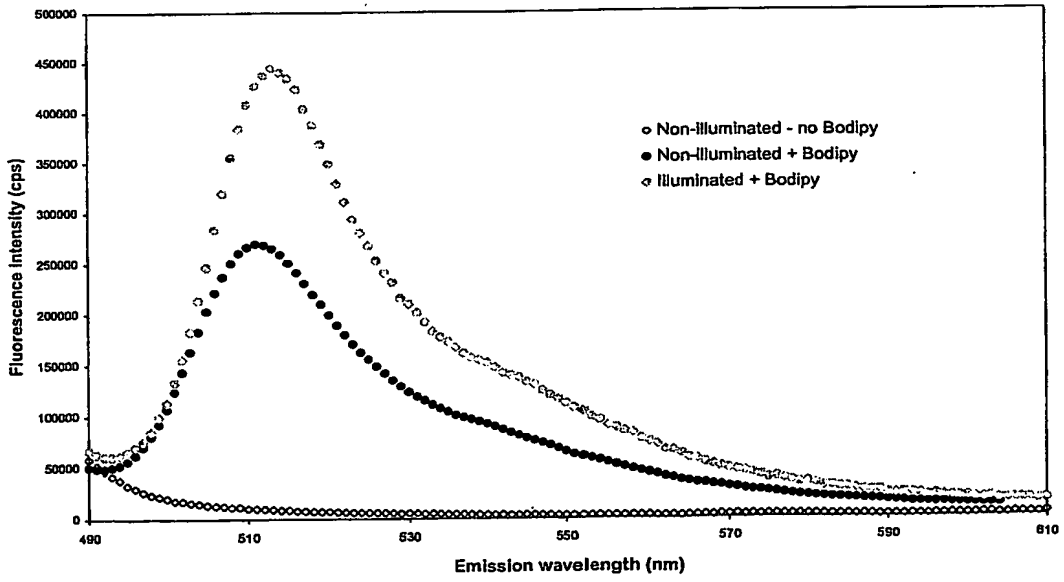

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence intensity (cps)
490
510
530
550
570
590
610
Emission wavelength (nm)
Non-illuminated - no Bodipy
Non-illuminated + Bodipy
Illuminated + Bodipy

Figure 9

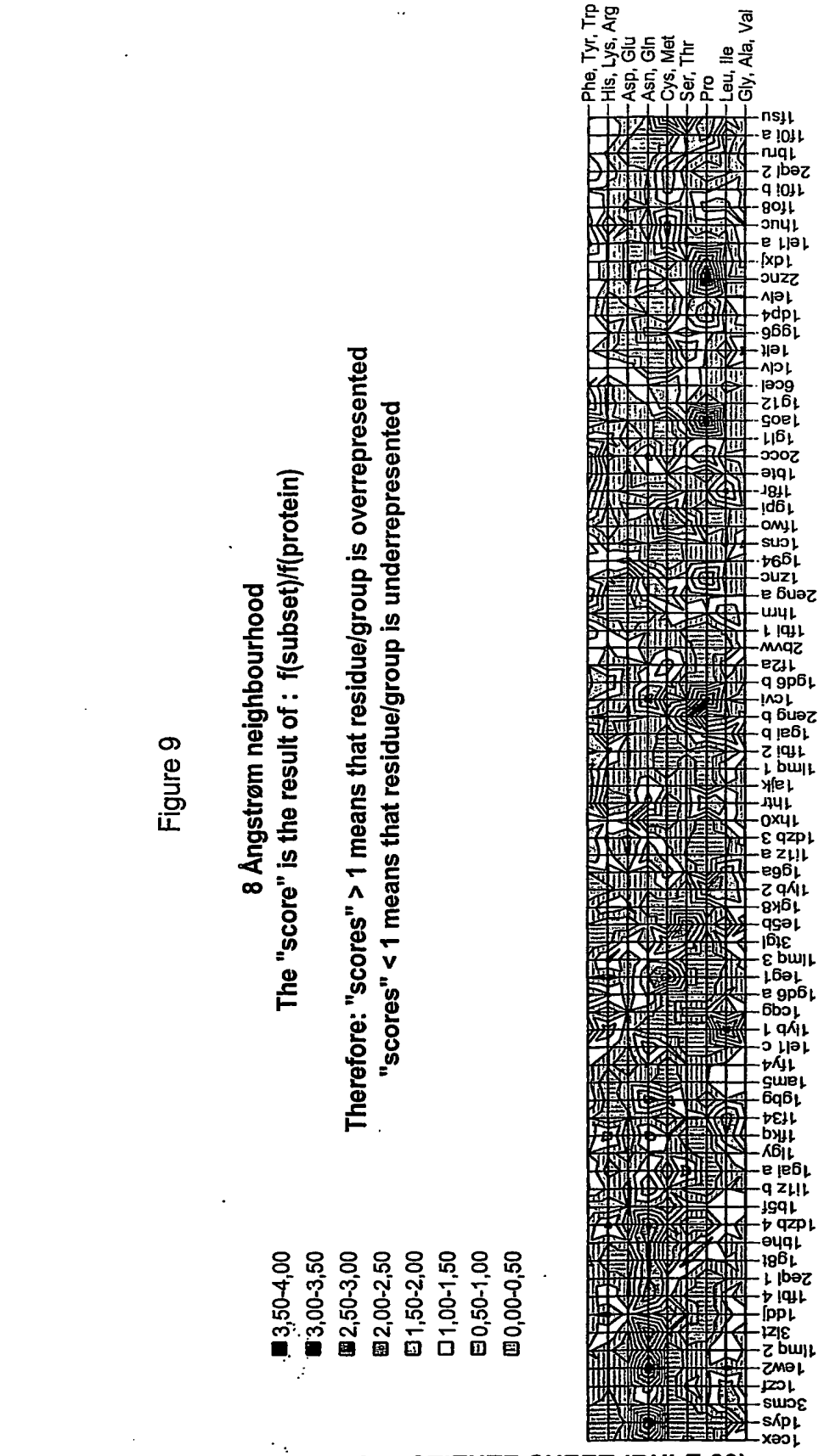

8 Ångstrøm neighbourhood
The "score" is the result of : f(subset)/f(protein)
Therefore: "scores" > 1 means that residue/group is overrepresented
"scores" < 1 means that residue/group is underrepresented
3,50-4,00
3,00-3,50
2,50-3,00
2,00-2,50
1,50-2,00
1,00-1,50
0,50-1,00
0,00-0,50
Phe, Tyr, Trp
His, Lys, Arg
Asp, Glu
Asn, Gln
Cys, Met
Ser, Thr
Pro
Leu, Ile
Gly, Ala, Val
1cex
1dys
3cms
1ccf
1ew2
1lmq 2
3izl
1ddj
1fbi 4
2eql 1
1g8t
1bhe
1dzb 4
1b5f
1i1z b
1gai a
1igy
1fkq
1f34
1gbg
1am5
1iy4
1el1 c
1iyb 1
1cqg
1gd6 a
1eg1
1lmq 3
3tgl
1esb
1gk8
1iyb 2
1g6a
1i1z a
1dzb 3
1hx0
1htr
1ajk
1lmq 1
1fbi 2
1gai b
2eng b
1cvl
1gd6 b
1f2a
2bvw
1fbi 1
1hrn
2eng a
1zrc
1g94
1cns
1fwo
1gpl
1f8r
1ble
2occ
1g11
1ao5
1g12
6cel
1clv
1elt
1gg6
1dp4
1elv
2znc
1dxj
1el1 a
1huc
1f08
1f0l b
2eql 2
1bru
1f0l a
1fsu

Figure 10

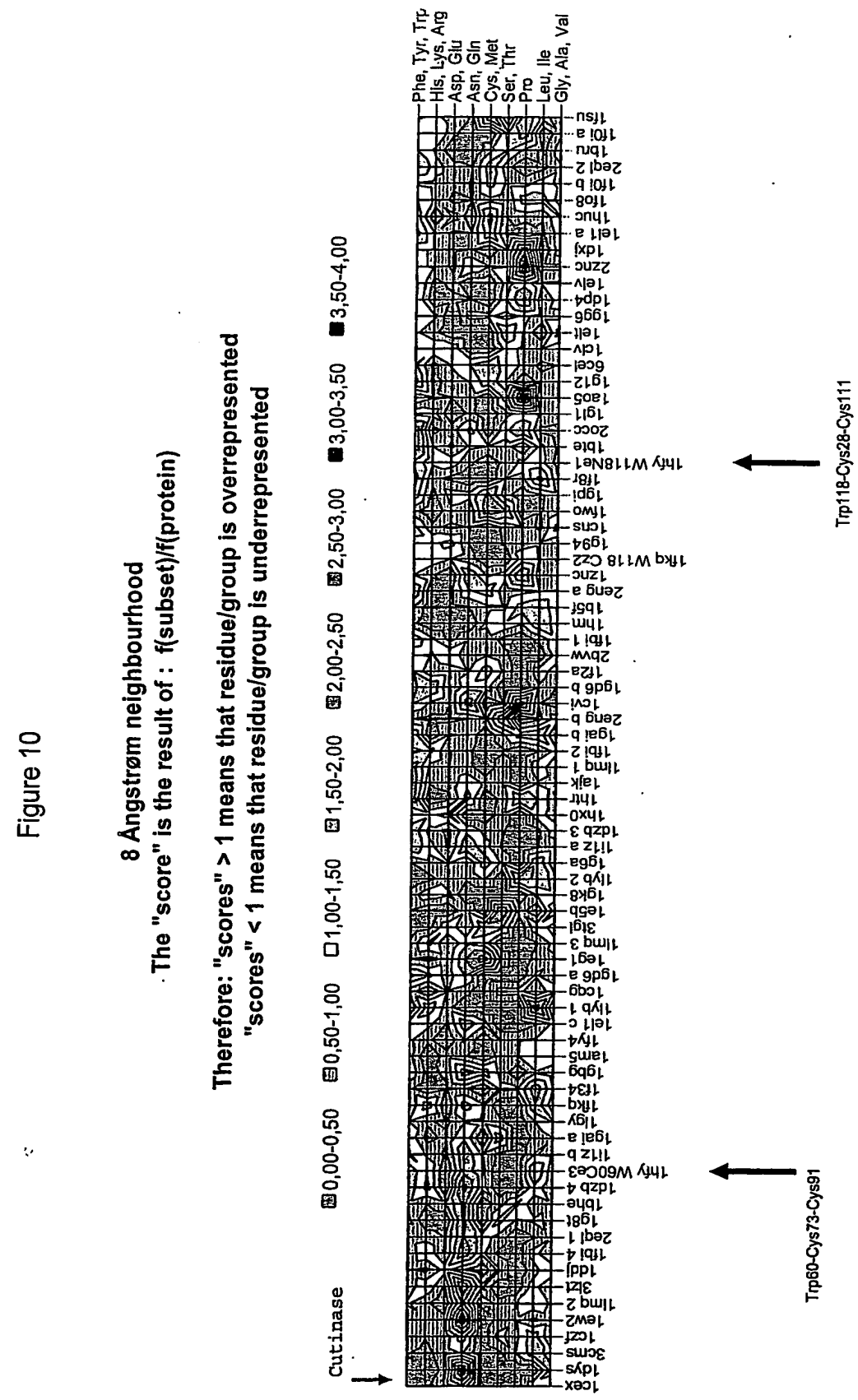

8 Ångstrøm neighbourhood
The "score" is the result of : f(subset)/f(protein)
Therefore: "scores" > 1 means that residue/group is overrepresented
"scores" < 1 means that residue/group is underrepresented
0,00-0,50
0,50-1,00
1,00-1,50
1,50-2,00
2,00-2,50
2,50-3,00
3,00-3,50
3,50-4,00
Cutinase
Phe, Tyr, Trp
His, Lys, Arg
Asp, Glu
Asn, Gln
Cys, Met
Ser, Thr
Pro
Leu, Ile
Gly, Ala, Val
1hfy W60Ce3
1hfy W118Ne1
1rkq W118 Cz2
Trp60-Cys73-Cys91
Trp118-Cys28-Cys111

Figure 11

8 Ångstrøm neighbourhood
The triad of cutinase compared to IGG1 triads A)

The "score" is the result of : f(subset)/f(protein)

Therefore: "scores" > 1 means that residue/group is overrepresented
"scores" < 1 means that residue/group is underrepresented

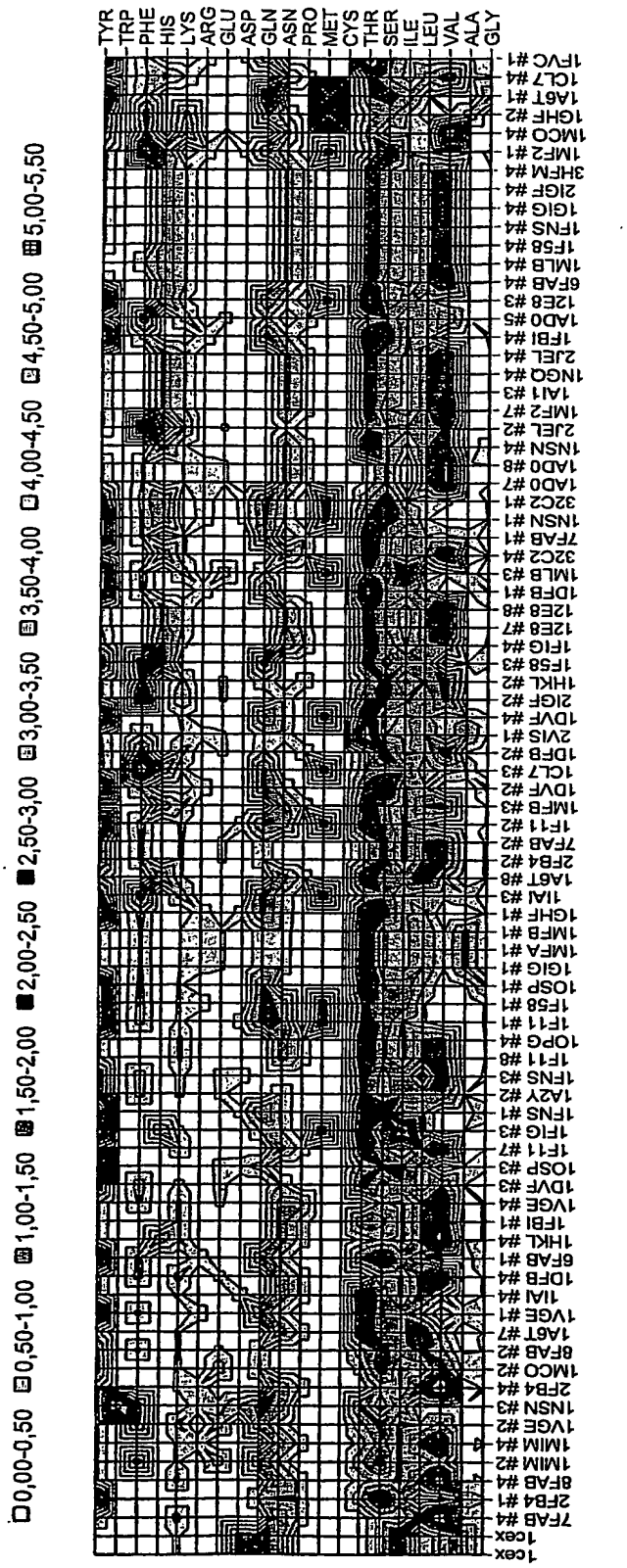


Figure 12

8 Ångstrøm neighbourhood
The triad of cutinase compared to IGG1 triads B)

The "score" is the result of : f(subset)/f(protein)

Therefore: "scores" > 1 means that residue/group is overrepresented
"scores" < 1 means that residue/group is underrepresented

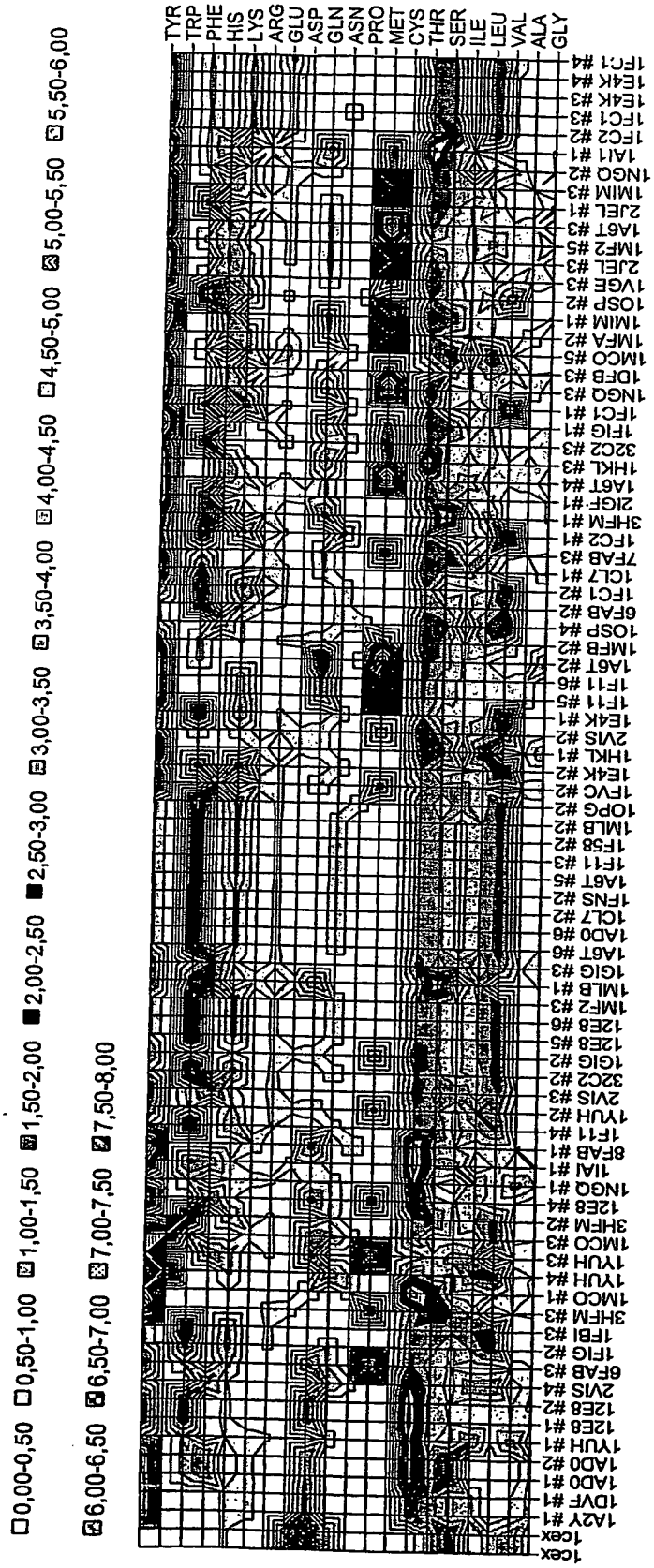


Figure 13

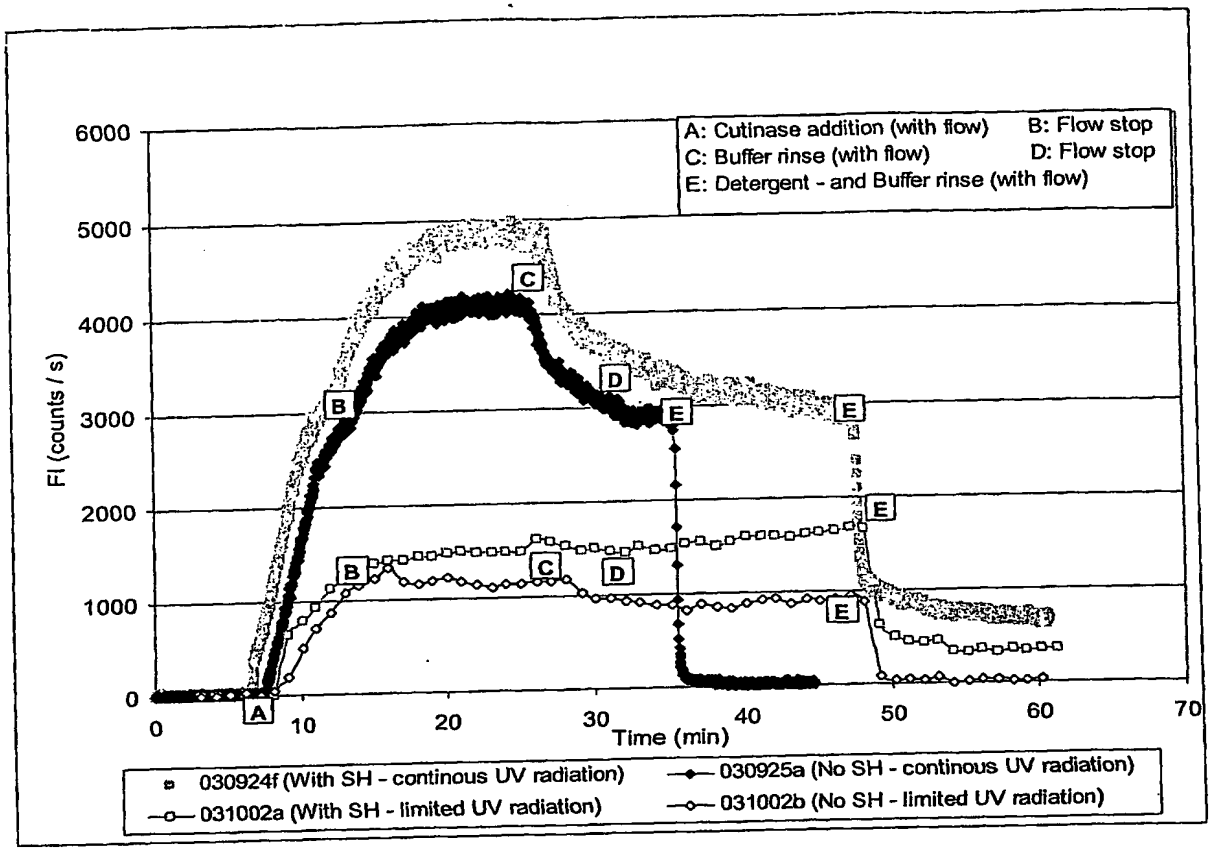
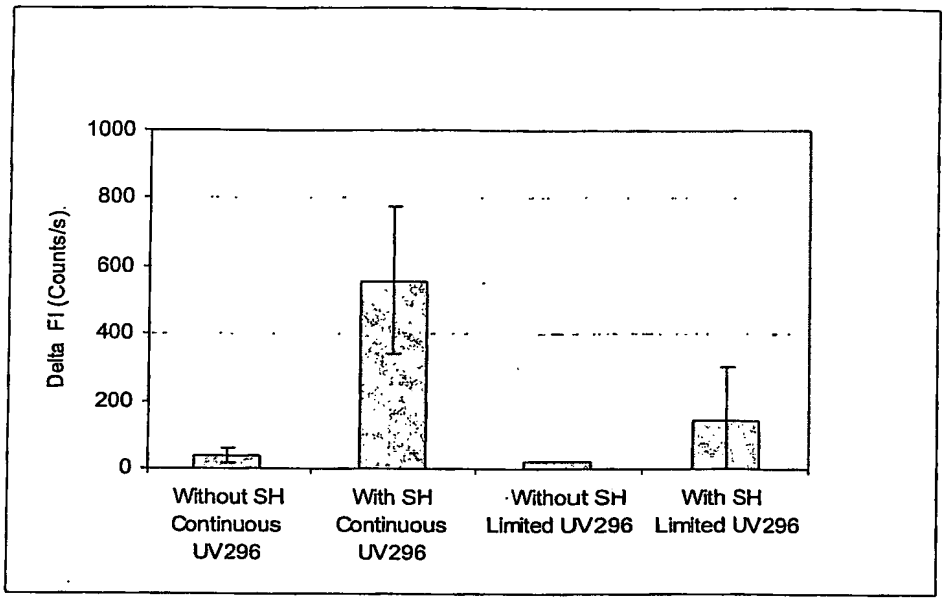

A: Cutinase addition (with flow)
B: Flow stop
C: Buffer rinse (with flow)
D: Flow stop
E: Detergent - and Buffer rinse (with flow)
FI (counts / s)
Time (min)
030924f (With SH - continous UV radiation)
030925a (No SH - continous UV radiation)
031002a (With SH - limited UV radiation)
031002b (No SH - limited UV radiation)

Figure 14

A


Delta FI (Counts/s).
1000
800
600
400
200
0
Without SH Continuous UV296
With SH Continuous UV296
Without SH Limited UV296
With SH Limited UV296

B

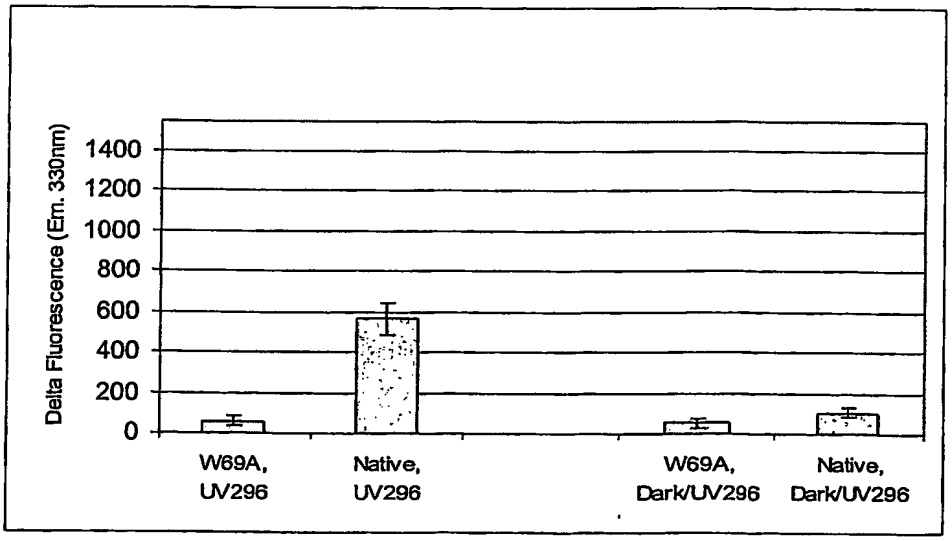

Delta Fluorescence (Em. 330nm)
1400
1200
1000
800
600
400
200
0
W69A, UV296
Native, UV296
W69A, Dark/UV296
Native, Dark/UV296

Figure 15

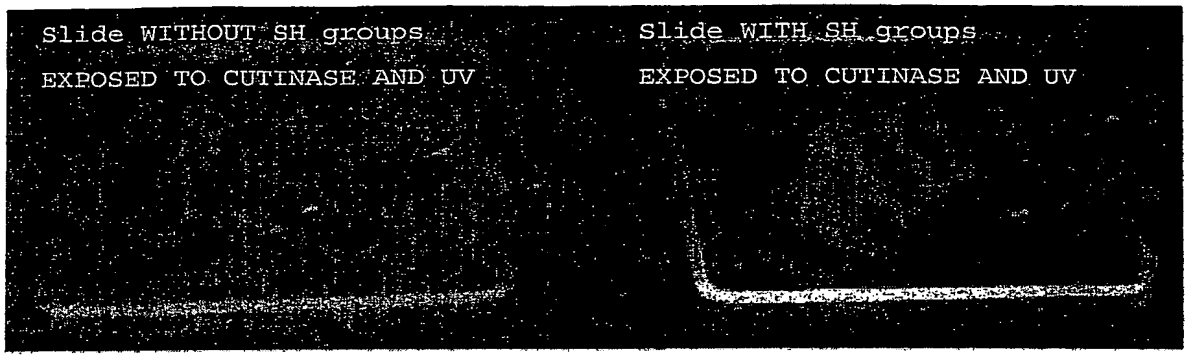
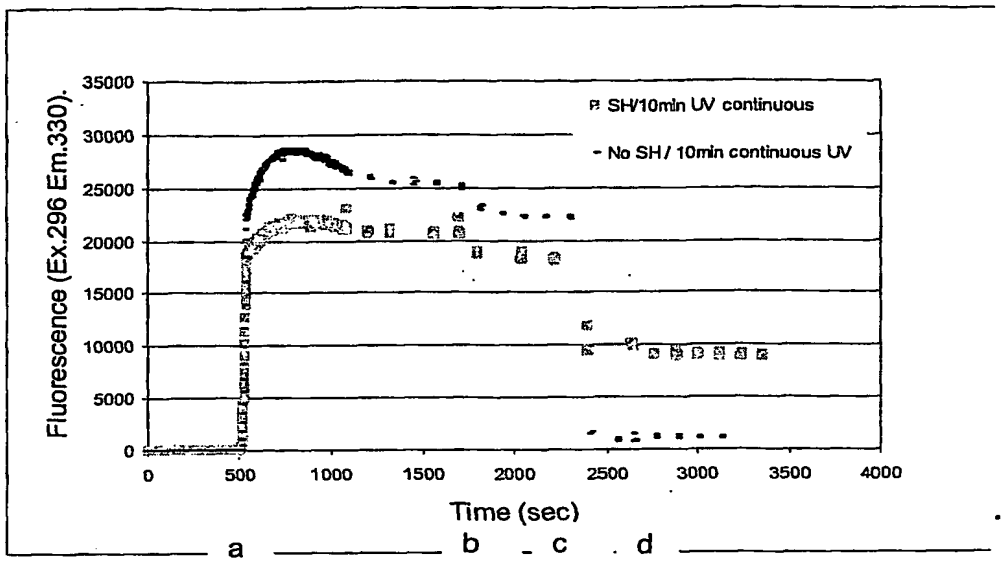
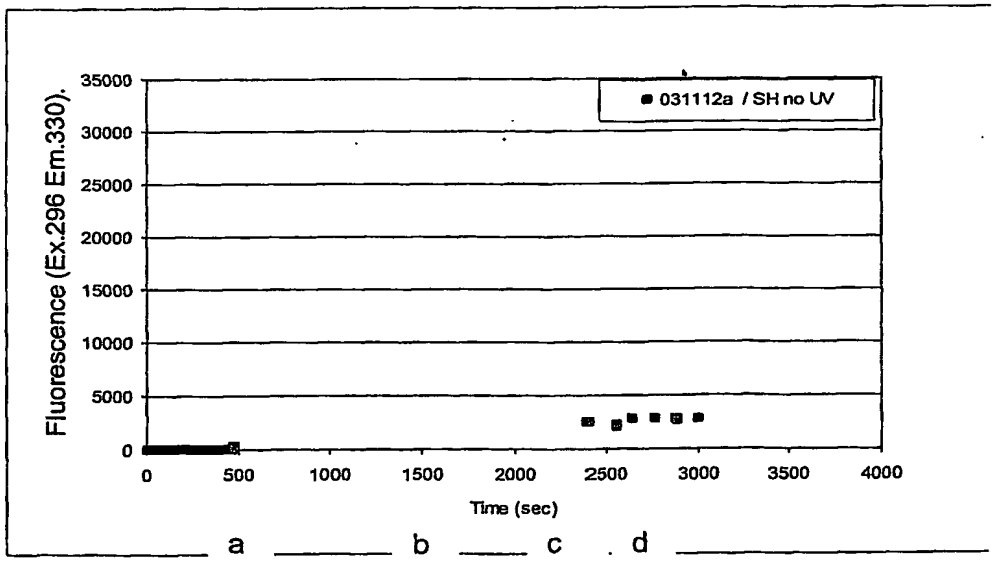

Slide WITHOUT SH groups
EXPOSED TO CUTINASE AND UV
Slide WITH SH groups
EXPOSED TO CUTINASE AND UV

Figure 16

A


Fluorescence (Ex.296 Em.330).
35000
30000
25000
20000
15000
10000
5000
0
0
500
1000
1500
2000
2500
3000
3500
4000
Time (sec)
SH/10min UV continuous
No SH / 10min continuous UV
a
b
c
d

B


Fluorescence (Ex.296 Em.330).
35000
30000
25000
20000
15000
10000
5000
0
0
500
1000
1500
2000
2500
3000
3500
4000
Time (sec)
031112a / SH no UV
a
b
c
d

C

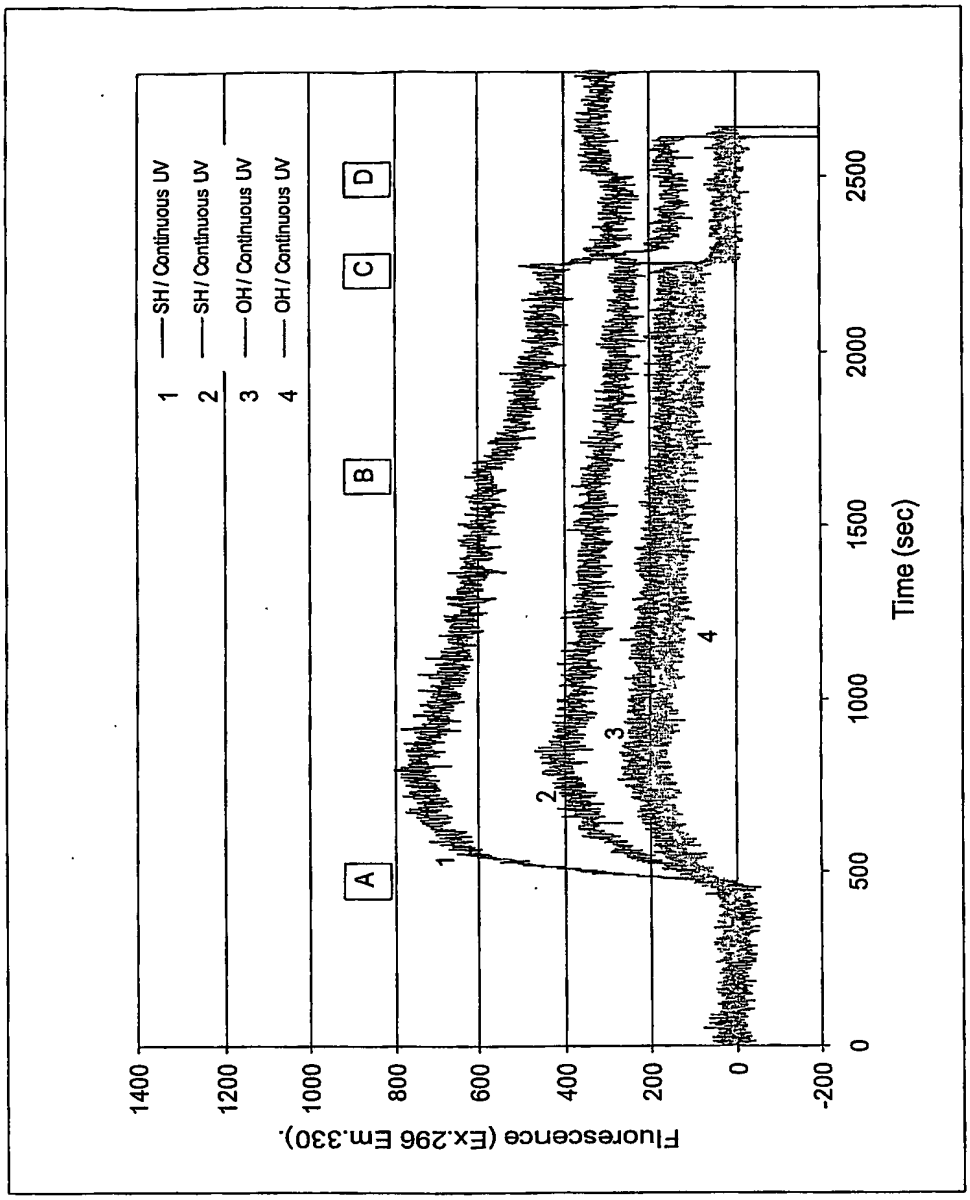

1 —SH / Continuous UV
2 —SH / Continuous UV
3 —OH / Continuous UV
4 — OH / Continuous UV
A
B
C
D
1
2
3
4
Fluorescence (Ex.296 Em.330).
Time (sec)
1400
1200
1000
800
600
400
200
0
-200
0
500
1000
1500
2000
2500

Figure 17 A

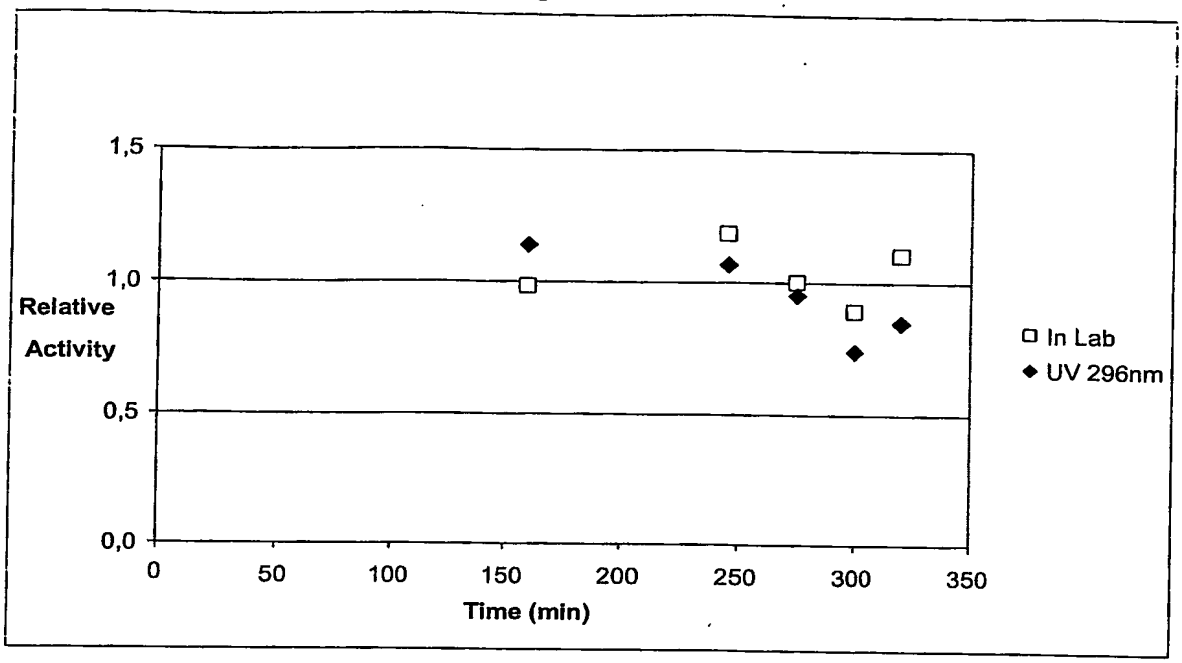
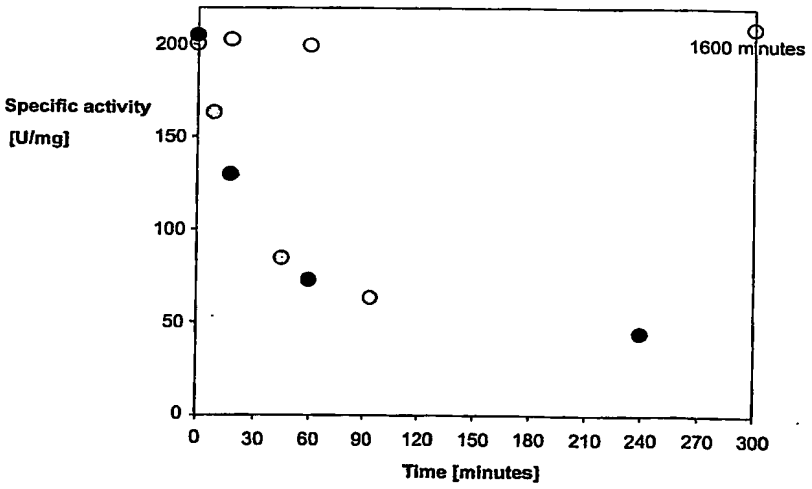



Figure 17B